

## **Section 4: Identification of Hallucinogenic Mushrooms**

### *I. Introduction:*

Hallucinogenic mushrooms are first given a visual test to check for their characteristic bluish-green striations in the stems. Then the mushroom sample are solvent extracted using Ethyl Ether. This extract is then run on the GC/MS. If the mushroom sample is a hallucinogen, psilocin will be detected on the GC/MS.

### *II. Reagents:*

- A.) 3 N Acetic Acid (HOAC): 37.5 mL glacial acetic acid in 412.5 mL distilled water.
- B.) Ethyl Ether
- C.) Ammonium Hydroxide (NH<sub>4</sub>OH).
- D.) Methanol
- E.) Anhydrous Sodium Sulfate (Na<sub>2</sub>SO<sub>4</sub>).

### *III. Equipment:*

- A.) 250 mL glass beakers (4).
- B.) Mortar and pestle.
- C.) 7.0 cm filter paper.
- D.) 250 mL volumetric vacuum flask.
- E.) Glass separatory funnels with stoppers and stopcocks (2).
- F.) Glass stir rod.
- G.) pH paper.
- H.) Glass pipettes.
- I.) Hot sand bath.
- J.) 5 uL autosampler syringe (for manual injections)
- K.) GC/MS: HP 5890/5972 series.

### *IV. Procedure:*

#### A.) Extraction Using Ethyl Ether:

1. Observe stems of the mushroom sample for bluish-green striations.
2. Weigh out 1.0 g of sample in a tared 250 mL beaker. Be sure to include all parts of the mushroom (caps and stems).
3. Grind the sample into finer pieces with a mortar and pestle. Add back to the beaker.
4. Fill beaker with 3 N HOAC so solution is just above the sample and so sample is saturated. Let sit overnight in a dark place (ex. drawer) because light will break down the psilocin. For quicker extractions, the beaker may be heated at 80° for one hour.
5. Vacuum filter with 3 N HOAC, making sure to rinse the beaker very well.

6. In a hood, extract with ethyl ether by pouring the filtered sample into a glass separatory funnel, add ethyl ether (5:1 organic to aqueous), stopper and invert a several times. Make sure to vent the funnel to let excess gas inside the funnel escape.
7. Remove stopper and allow the layers to separate. The ethyl ether layer will be on the top.
8. Drain the bottom layer directly into another glass separatory funnel and discard the ethyl ether layer into appropriate waste container.
9. Add  $\text{NH}_4\text{OH}$  until solution is basic.
10. Extract again with ethyl ether (5:1 organic to aqueous). This time discard the bottom layer into waste container and drain the ethyl ether layer through a funnel filled with Anhydrous  $\text{Na}_2\text{SO}_4$  (to remove any water) into a beaker labeled with the sample number.
11. Either let the sample evaporate overnight in a dark place, or place on a hot sand bath until dry.
12. Rinse the beaker with Methanol, swirling to make sure the sides of the beaker get hit with Methanol.

B.) Chromatography by GC/MS:

1. Manually inject, in the following order, 1.5 uL of the psilocin standard, blank, and sample(s) directly from the beaker into the GC/MS.
2. GC/MS conditions:  
 Method: PSILM.M  
 Oven:  
     Initial Temp: 230°C  
     Initial Time: 0.00 min.  
     Max. Temp: 325°C  
     Equilibration Time: 0.50 min.  
     Rate: 10°/min.  
     Final Temp: 280°C  
     Run Time: 10 min.  
 Inlet:  
     Mode: split (50:1)  
     Initial Temp: 250°C  
     Pressure: 31.65 psi  
     Gas Type: Helium  
 Column:  
     Capillary: HP-1MS 25m x 200um x 0.33um  
     Max. Temp: 300°C  
     Initial Flow: 1.0 mL/min.
3. If psilocin is present in sample, the instrument will detect a total ion peak at its retention time and will generate a report along with accompanying chromatograph and spectra. The spectra will contain the identity of the peak and its ion abundance (see graph, last page).

*V. Results:*

- A.) Record results of the GC/MS in logbook. Then transfer the results to the Drug Lab Results sheet that came with the samples. Be sure to include date of analysis, results, the number of tests performed per sample, and signature.
- B.) All reports generated from the instruments should be filed so that they may be accessed at a later date, if necessary.

